

We claim:

1. A recombinant Ca^{2+} dependent monoclonal antibody immunoreactive with an epitope in the activation peptide region of the heavy chain of Protein C defined by E D Q V D P R L I D G K (Sequence ID No. 1) in combination with calcium, where the antibody inhibits Protein C activation by thrombin-thrombomodulin.

2. The antibody of claim 1 comprising amino acid sequence selected from the group consisting of:

HPC4 V11
HPC4 V12
MGRLLSSSFL LIAPAYVLSQ VTLKESGPGI LQPSQLTLT CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence ID No. 10); MDFQVQIFSF LLISASVIMS RGQIILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTSPKLLIY GTSNLASGVP SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR - (Sequence ID No. 12); Q VTLKESGPGI LQPSQLTLT CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (amino acids 20-139 of Sequence ID No. 10) and QIILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTSPKLLIY GTSNLASGVP SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR (amino acids 23-129 of Sequence ID No. 12).

3. The antibody of claim 1 containing human amino acid sequence.

4. The antibody of claim 1 encoded in part by a nucleotide sequence selected from the group consisting of ATGGGCAGGC TTTCTCTTC ATTCTTGCTA CTGATTGCCCT CTGCATATGT CCTGTCCAG GTTACTCTGA AAGAGTCTGG CCCTGGGATA TTGCAGCCCT CCCAGACCT CACTCTGACT TGTTCTCTCT CTGGGTTTTC ACTGAGGACT TCTGGTATGG GTGTAGGCTG GATTGTCAG CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA CACATTTGGT GGGATGATGA CAAGCGCTAT AACCCAGTCC TGAAGAGCCG ACTGATAATC TCCAAGGATA

(Sequence ID
No. 9); CAG GTTACTCTGA AAGAGTCTGG CCCTGGGATA
TTGCAGCCCT CCCAGACCCCT CACTCTGACT TGTTCCTCTCT CTGGGTTTTC
ACTGAGGACT TCTGGTATGG GTGTAGGCTG GATTCGTCAG CCTTCAGGGA
AGGGTCTGGA GTGGCTGGCA CACATTTGGT GGGATGATGA CAAGCGCTAT
AACCCAGTCC TGAAGAGCCG ACTGATAATC TCCAAGGATA CCTCCAGGAA
ACAGGTATTCTCACTCAAGATCG CCAGTGTGGAA CACTGCAGAT ACTGCCACAT
ACTACTGTGT TCGAATGATG GATGATTACG ACGCTATGGA CTACTGGGTT
CAAGGAACCT CAGTCACCGT CTCCCTCT (nucleotides 58 to 417
of Sequence ID No. 9); ATGGATTTC AGGTGCAGAT
TTTCAGCTTC CTGCTAATCA GTGCCTCAGT CATAATGTCC AGAGGACAAA
TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT GGGGGAGGAG
ATCACCCCTAA CCTGCAGTGC CACTTCGAGT GTAACTTACG TCCACTGGTA
CCAGCAGAAG TCAGGCACCT CTCCCCAAACT CTTGATTTAT GGGACATCCA
ACCTGGCTTC TGGAGTCCCT TCTCGTTCA GTGGCAGTGG
GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG GCTGAAGATG
CTGCCGATTA TTACTGCCAT CAGTGGAAATA GTTATCCGCA CACGTTCGGA
GGGGGGACCA AGCTGGAAAT AAAACGG (Sequence ID No. 11); - HPLC-4 V
CAAATTTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT
GGGGGAGGAG ATCACCCCTAA CCTGCAGTGC CACTTCGAGT GTAACTTACG
TCCACTGGTA CCAGCAGAAG TCAGGCACCT CTCCCCAAACT CTTGATTTAT
GGGACATCCA ACCTGGCTTC TGGAGTCCCT TCTCGTTCA
GTGGCAGTGG GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG
GCTGAAGATG CTGCCGATTA TTACTGCCAT CAGTGGAAATA GTTATCCGCA
CACGTTCGGA GGGGGGACCA AGCTGGAAAT AAAACGG (nucleotides
67 to 387 of Sequence ID No. 11); and degenerate
sequences thereof.

Sub A 2

5. The antibody of claim 1 further
comprising a pharmaceutically acceptable carrier for
administration to a patient.

6. The antibody of claim 5 further
comprising a cytokine or an inducer of cytokine
expression in a dosage effective in combination with

the antibody to coagulate microvasculature in tumors but not in the absence of the antibody.

Sub C2 7. The antibody of claim 1 having a detectable label bound to the antibody.

Sub A3 8. The antibody of claim 1 immobilized to a substrate, wherein the immobilized antibody is suitable for purification of protein C from a biological fluid.

9. A method for treating a disorder by inhibition of protein C anticoagulant comprising administering to a patient in need of treatment thereof an effective amount of a recombinant Ca^{2+} dependent monoclonal antibody immunoreactive with an epitope in the activation peptide region of the heavy chain of Protein C defined by E D Q Y D P R L I D G K (Sequence ID No. 1) in combination with calcium, where the antibody inhibits Protein C activation by thrombin-thrombomodulin.

10. The method of claim 9 wherein the antibody comprises amino acid sequence selected from the group consisting of:

MGRLLSSSFLL LIAPAYVLSQ VTLKESGPGI LQPSQTTLT CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence ID No. 10); MDFQVQIFS LLISASVIMS RGQIILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTPSKLLIY GTSNLASGVP SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR (Sequence ID No. 12); Q VTLKESGPGI LQPSQTTLT CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (amino acids 20-139 of Sequence ID No. 10) and QIILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTPSKLLIY GTSNLASGVP SRFSGSGSGT FYSLTVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR (amino acids 23-129 of Sequence ID No. 12).

11. The method of claim 9 wherein the antibody contains human amino acid sequence.

12. The method of claim 9 wherein the antibody is encoded in part by a nucleotide sequence selected from the group consisting of ATGGGCAGGC
TTTCTTCTTC ATTCTTGCTA CTGATTGCCCT GCATATGT CCTGTCCCAG
GTTACTCTGA AAGAGTCTGG CCCTGGGATA TTGCAGCCCT CCCAGACCCT
CACTCTGACT TGTTCTCTCT CTGGGTTTC ACTGAGGACT TCTGGTATGG
GTGTAGGCTG GATTGTCAG CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA
CACATTGGT GGGATGATGA CAAGCGCTAT AACCCAGTCC TGAAGAGCCG
ACTGATAATC TCCAAGGATA CCTCCAGGAA ACAGGTATTCT CTCAGATCG
CCAGTGTGGA CACTGCAGAT ACTGCCACAT ACTACTGTGT TCGAATGATG
GATGATTACG ACGCTATGGA CTACTGGGTT CAAGGAACCT CAGTCACCGT
CTCCTCT (Sequence ID No. 9); CAG GTTACTCTGA AAGAGTCTGG
CCCTGGGATA TTGCAGCCCT CCCAGACCCT CACTCTGACT TGTTCTCTCT
CTGGGTTTC ACTGAGGACT TCTGGTATGG GTGTAGGCTG GATTGTCAG
CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA CACATTGGT GGGATGATGA
CAAGCGCTAT AACCCAGTCC TGAAGAGCCG ACTGATAATC TCCAAGGATA
CCTCCAGGAA ACAGGTATTCT CTCAGATCG CCAGTGTGGA CACTGCAGAT
ACTGCCACAT ACTACTGTGT TCGAATGATG GATGATTACG ACGCTATGGA
CTACTGGGTT CAAGGAACCT CAGTCACCGT CTCCTCT (nucleotides
58 to 417 of Sequence ID No. 9); ATGGATTTTC AGGTGCAGAT
TTTCAGCTTC CTGCTAXTCA GTGCCTCAGT CATAATGTCC AGAGGACAAA
TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT GGGGGAGGAG
ATCACCCCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG TCCACTGGTA
CCAGCAGAAG TCAGGCACCT CTCCCAAACCT CTTGATTTAT GGGACATCCA
ACCTGGCTTC TGGAGTCCCT TCTCGTTCA GTGGCAGTGG
GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG GCTGAAGATG
CTGCCGATTA TTACTGCCAT CAGTGGAAATA GTTATCCGCA CACGTTCGGA
GGGGGGACCA AGCTGGAAAT AAAACGG (Sequence ID No. 11);
CAAA TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT
GGGGGGAGGAG ATCACCCCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG
TCCACTGGTA CCAGCAGAAG TCAGGCACCT CTCCCAAACCT CTTGATTTAT
GGGACATCCA ACCTGGCTTC TGGAGTCCCT TCTCGTTCA
GTGGCAGTGG GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG

GCTGAAGATG CTGCCGATTA TTACTGCCAT CAGTGGAAATA GTTATCCGCA
CACGTTCGGA GGGGGGACCA AGCTGGAAAT AAAACGG (nucleotides
67 to 387 of Sequence ID No. 11); and degenerate
sequences thereof.

13. The method of claim 9 further comprising administering with the antibody a cytokine or other chemotherapeutic agent in an amount effective to coagulate the microvasculature of a tumor.

14. A method of making a recombinant Ca^{2+} dependent monoclonal antibody immunoreactive with an epitope in the activation peptide region of the heavy chain of Protein C defined by E D Q V D P R L I D G K (Sequence ID No. 1) in combination with calcium, where the antibody inhibits Protein C activation by thrombin-thrombomodulin, by expressing nucleotide sequence encoding the antibody.

15. The method of claim 14 wherein the antibody comprises amino acid sequence selected from the group consisting of:

MGRLSSSFLL LIAPAYVLSQ VTLKESGPGI LQPSQTLTLT CSLSGFSLRT
SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII SKDTSRKQVF
LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence
ID No. 10); MDFQVQIFS LLISASVIMS RGQIILTQSP
AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTPSKLLIY GTSNLASGVP
SRFSGSGSGT FYSITVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR
(Sequence ID No. 12); Q VTLKESGPGI LQPSQTLTLT
CSLSGFSLRT SGMGVGWRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII
SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS
(amino acids 20-139 of Sequence ID No. 10) and
QIILTQSP AIMSASLGEE ITLTCSATSS VTYVHWYQQK SGTPSKLLIY
GTSNLASGVP SRFSGSGSGT FYSITVSSVE AEDAADYYCH QWNSYPHTFG
GGTKLEIKR (amino acids 23-129 of Sequence ID No. 12).

16. The method of claim 14 wherein the antibody is encoded in part by a nucleotide sequence

Sub
A 4

selected from the group consisting of ATGGGCAGGC
TTTCTTCTTC ATTCTTGCTA CTGATTGCC CTCATATGT CCTGTCCCAG
GTTACTCTGA AAGAGTCTGG CCCTGGGATA TTGCAGCCCT CCCAGACCCCT
CACTCTGACT TGTTCTCTCT CTGGGTTTC ACTGAGGACT TCTGGTATGG
GTGTAGGCTG GATTCGTCAG CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA
CACATTTGGT GGGATGATGA CAAGCGCTAT AACCCAGTOC TGAAGAGCCG
ACTGATAATC TCCAAGGATA CCTCCAGGAA ACAGGTATTG CTCAAGATCG
CCAGTGTGGA CACTGCAGAT ACTGCCACAT ACTACTGTGT TCGAATGATG
GATGATTACG ACGCTATGGA CTACTGGGTT CAAGGAACCT CAGTCACCGT
CTCCTCT (Sequence ID No. 9); CAG GTTACTCTGA AAGAGTCTGG
CCCTGGGATA TTGCAGCCCT CCCAGACCCCT CACTCTGACT TGTTCTCTCT
CTGGGTTTC ACTGAGGACT TCTGGTATGG GTGTAGGCTG GATTCGTCAG
CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA CACATTTGGT GGGATGATGA
CAAGCGCTAT AACCCAGTCC TGAAGAGCCG ACTGATAATC TCCAAGGATA
CCTCCAGGAA ACAGGTATTG CTCAAGATCG CCAGTGTGGA CACTGCAGAT
ACTGCCACAT ACTACTGTGT TCGAATGATG GATGATTACG ACGCTATGGA
CTACTGGGTT CAAGGAACCT CAGTCACCGT CTCCTCT (nucleotides
58 to 417 of Sequence ID No. 9); ATGGATTTTC AGGTGCAGAT
TTTCAGCTTC CTGCTAACGTA GTGCCTCACT CATAATGTCC AGAGGACAAA
TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT GGGGGAGGAG
ATCACCCCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG TCCACTGGTA
CCAGCAGAAG TCAGGCACCT CTCCCAAACCT CTTGATTTAT GGGACATCCA
ACCTGGCTTC TGGAGTCCCT TCTCGTTCA GTGGCAGTGG
GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG GCTGAAGATG
CTGCCGATTA TTACTGCCAT CAGTGGAAATA GTTATCCGCA CACGTTCGGA
GGGGGGACCA AGCTGGAAAT AAAACGG (Sequence ID No. 11);
CAAA TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT
GGGGGGAGGAG ATCACCCCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG
TCCACTGGTA CCAGCAGAAG TCAGGCACCT CTCCCAAACCT CTTGATTTAT
GGGACATCCA ACCTGGCTTC TGGAGTCCCT TCTCGTTCA
GTGGCAGTGG GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG
GCTGAAGATG CTGCCGATTA TTACTGCCAT CAGTGGAAATA GTTATCCGCA
CACGTTCGGA GGGGGGACCA AGCTGGAAAT AAAACGG (nucleotides
67 to 387 of Sequence ID No. 11); and degenerate
sequences thereof.

Sub A 57
17. The method of claim 14 further comprising inserting human sequence into the antibody in place of animal sequence.

Sub C57
18. The method of claim 14 further comprising binding detectable label to the antibody.

Sub A 7
19. The method of claim 14 further comprising immobilizing the antibody to a substrate, wherein the immobilized antibody is suitable for purification of protein C from a biological fluid.

Add A 1